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EFFECT OF LIGHT SPECTRUM ON THE PHYCOCYANIN PRODUCTION BY *ARTHROSPIRA PLATENSIS*

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Abstract

The global market shows high demand for products of natural origin to reduce the use of synthetic substances in the industries. One of the opportunities for acquiring natural compounds of industrial value is the use of cyanobacteria biomass. In terms of biomass composition, cyanobacteria of the species *Arthrospira platensis*, which contains phycocyanin, deserve special attention. Phycocyanin is a pigment-protein complex widely used in the food and cosmetics industries, histochemistry, fluorescence microscopy and flow cytometry. The high demand for this pigment determines the search for methods to intensify phycocyanin production by *A. platensis*. The aim of the study was to determine the effect of light of different wavelengths on phycocyanin productivity by cyanobacteria of the species *Arthrospira platensis*. The highest biomass concentration and biomass production efficiency were obtained in a culture using Blue-Red LED lighting and they amounted to 5.619 ± 0.053 g TS dm^{-3} and 656 ± 7 mg dm^{-3} d^{-1}. The highest phycocyanin concentration and purity were observed in a culture using Red LED lighting and they amounted to 17.61 ± 0.51% TS and 0.710 ± 0.01.

Introduction

The global market shows high demand for products of natural origin to reduce the use of synthetic substances in the food, pharmaceutical and cosmetics industries. This has led to a growing interest in biotechnological research focused on increasing the rate and efficiency of acquiring natural products through the elimination of the main limiting factors, such as e.g. crop seasonality. One of the opportunities for acquiring natural compo-

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unds of industrial value is the use of cyanobacteria biomass that exhibit high biomass productivity and can be cultured in photobioreactors, which restricts the impact of external conditions on the culture and hinders the access of both parasites and competing microorganism species (KIM et al. 2013). In terms of biomass composition, cyanobacteria of the species *Arthrospira platensis* deserve particular attention. They are characterised by high contents of protein, γ-linolenic acid (COHEN 1997), polysaccharides (De PHILIPPIS and VINCENZINI 1988), β-carotene (GIREESH et al. 2001), chlorophyll and phycocyanin (RANGEL-YAGUI et al. 2004). The species *Arthrospira platensis* is cultured on a commercial scale for the extraction of phycocyanin, a compound with a high added value which is used in a variety of industries.

In the *A. platensis* cells, this compound is used as the main photoreceptor in the photosynthesis process and is found, along with other phycobiliproteins, in complexes referred to as phycobilisomes (GANTT 1981, MIMURO and KIKUCHI 2003). It is estimated that the phycocyanin content of the *Arthrospira platensis* cells may be as high as 15% of the dry matter (WAN et al. 2016).

Phycocyanin is a pigment-protein complex used in food products to increase nutritional value. It is used as food colourants, antioxidants and emulsifiers which can replace or reduce the use of synthetic additives. The substance is also widely used as a pigment in the cosmetics industry and as a fluorescent biomarker in laboratory testing (ZHAO et al. 2014). The pigment is used as fluorescent probes in histochemistry, fluorescence microscopy, flow cytometry and fluorescence immunoassay (SEKAR and CHANDRAMOHAN 2008).

Photosynthesising organisms such as *Arthrospira platensis* can be cultivated using advanced technologies enabling thorough monitoring and controlling the conditions as well as in open ponds (DĘBOWSKI et al. 2012). In closed systems, photobioreactors are used, which, unlike open cultures, enable continuous monitoring of the temperature and the pH of the culture medium and the control of the method, intensity and duration of lighting (KAEWPINTONG et al. 2007, PULZ 2001). The provision of adequate lighting is one of the most important factors affecting the biomass productivity and the content of assimilation pigments. Scientific research has shown that the type of light source and the wavelength not only affect biomass productivity but also the chemical composition of cyanobacteria (HO et al. 2014a, HO et al. 2014b). Compared to traditional fluorescent lamps, light-emitting diodes (LED) characterised by narrow-band wavelength, low energy consumption and high reliability are regarded as the optimal light source for the cultivation of photosynthesising organisms (SCHULZE et al. 2014).

The aim of the study was to determine the effect of light of different wavelengths on phycocyanin productivity by cyanobacteria of the species *Arthrospira platensis*.

Materials and Methods

Microorganism Strain and Culture Medium

The biomass of cyanobacteria *Arthrospira platensis* used in the experiment originates from a culture carried out under controlled conditions, initiated from a culture acquired from the Experimental Phycology and Culture Collection of Algae Centre in Göttingen (Fig. 1).

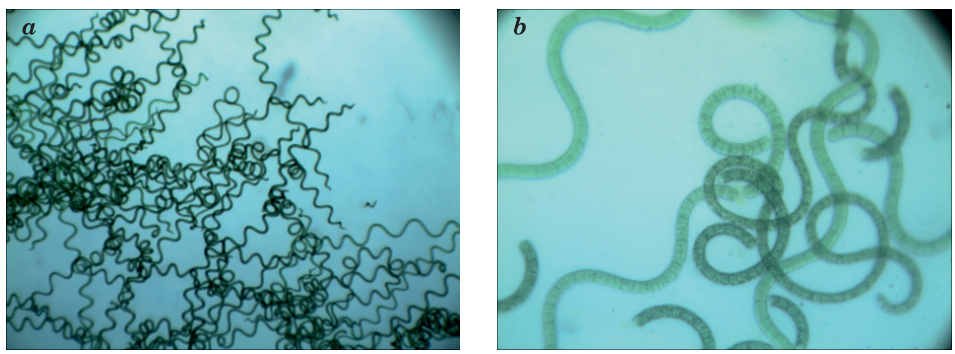

Fig. 1. *Arthrospira platensis* seen in microscope, magnified: *a* – 100x; *b* – 40x

In the experimental culture, a modified medium AIBA and OGAWA was used (Table 1) (AIBA and OGAWA 1977).

Table 1

The composition of the culture medium used in the experiment

Component	Unit	Amount
$NaHCO_3$	g dm^{-3}	13.61
Na_2CO_3	g dm^{-3}	4.03
K_2HPO_4	g dm^{-3}	0.50
$NaNO_3$	g dm^{-3}	2.50
K_2SO_4	g dm^{-3}	1.00
NaCl	g dm^{-3}	1.00
$MgSO_4 \cdot 7\ H_2O$	g dm^{-3}	0.20
$CaCl_2 \cdot 2\ H_2O$	g dm^{-3}	0.04
$FeSO_4 \cdot 7\ H_2O$	g dm^{-3}	0.014

EDTA (Titriplex III, MERC)	g dm^{-3}	0.084
$ZnSO_4 \cdot 7\ H_2O$	µg dm^{-3}	5
$MnSO_4 \cdot 4\ H_2O$	µg dm^{-3}	10
H_3BO_3	µg dm^{-3}	50
$Co(NO_3)2 \cdot 6\ H_2O$	µg dm^{-3}	5
$Na_2MoO_4 \cdot 2\ H_2O$	µg dm^{-3}	5
$CuSO_4 \cdot 5\ H_2O$	µg dm^{-3}	0.005

Culture Condition

Arthrospira platensis were cultivated in pipe photobioreactors with a vertical orientation and an active volume of 2 dm^3. The temperature of the culture was 30 ± 1°C. The test stand was equipped with an aeration pump connected to the reactors from below. This solution supplied carbon dioxide from atmospheric air to the system and mixed the cyanobacteria culture. The volumetric aeration ratio was 0.6 v/v. The cultivation time was 8 days, then physicochemical analyzes were performed.

Light Sources

The study was divided into four experimental series with the light source as the division criterion:

– red-red LED (660 and 630 nm),
– blue-blue LED (470 and 430 nm),
– blue-red-blue-red LED (660, 630, 470 and 430 nm),
– white-white fluorescence lamp (5600 K).

In the experimental series using LED lighting, modules comprised of 1 Watt Helixeon diodes were used (Helio Opto, Taiwan). Each module comprised 28 diodes and a 36 W power supply unit (Philips, the Netherlands). In the control series using a fluorescent lamp, a 28 W lamp with a colour temperature of 5600 K was used (Osram, Germany). The lighting intensity in all series was about 7500 lux.

Measurement of Biomass Concentration

Dry matter content, or total solids (TS) was determined by filtering 50 ml of culture samples through a 110 mm diameter hard cellulose filter. Following the filtering process, the filter was dried in a laboratory oven (Binder, Germany) until a constant weight was obtained. In order to determine dry matter content, the difference between the weight of a dried empty filter and the weight of a dried filter following filtration was determined.

Determination of Growth Parameters and Phycocyanin Productivity

The biomass productivity (P_b, g TS dm^{-3} d^{-1}) was calculated based on the equation:

$$P_b = \frac{\Delta X}{\Delta t}$$

where:

ΔX – the difference in biomass concentration [g TS dm^{-3}] over a cultivation time of Δt (d).

Moreover, according to the mass balance of microalgae, the phycocyanin productivity ($P_{phy,}$ mg dm^{-3} d^{-1}) was calculated from the relationship between the phycocyanin content and volumetric growth rate of the microalgal cell, as indicated in the equation:

$$P_{\text{phy}} = P \cdot \text{EY}$$

where:

P – the biomass productivity [g TS dm^{-3} d^{-1}]

EY – the phycocyanin extraction yield [mg g^{-1} TS].

Phycocyanin extraction

To extract phycocyanin from the *Arthrospira platensis* cells, 0.5 g dry biomass was added to 50 cm^3 phosphate buffer (pH of 7.0). The prepared solution was subjected to ultrasonic disintegration. The ultrasonic treatment process was carried out using a 400 W UP400S ultrasonic processor with a frequency of 24 kHz (Hielscher, Germany). The ultrasound amplitude was 70%, and the disintegration time was 30 s. Following the ultrasonic disintegration process, the samples were shaken in the dark for 4 h. The samples were then centrifuged at 9,000 xg for 15 minutes and the supernatant was subjected to a spectrophotometric analysis.

Determination of Phycocyanin Concentration

After centrifugation, the supernatant's optical density was measured using a DR5000 spectrophotometer (Hach, USA). Phycocyanin content (PC, mg cm^{-3}) was calculated according to the following equation (BENNETT and BOGORAD 1973):

$$\text{PC} = \frac{\text{OD}_{615} - 0.474(\text{OD}_{652})}{5.34}$$

where:

OD_{615} – the optical density of the sample at 615 nm

OD_{652} – the optical density of the sample at 652 nm.

The purity index of phycocyanin is determined by dividing the phycocyanin maximum absorbance wavelength to a specific absorbance wavelength of total protein (OD_{615}/OD_{280}). The purity of phycocyanin extract (EP) was calculated according to the following equation (ABALDE et al. 1998):

$$EP = \frac{OD_{615}}{OD_{280}}$$

where:
OD_{615} – the optical density of the sample at 615 nm
OD_{280} – the optical density of the sample at 280 nm
indicating the total concentration of proteins in the solution.

The extraction yield (EY, mg g^{-1} TS^{-1}) was calculated as:

$$EY = \frac{PC \cdot V}{d_w}$$

where:
PC – the phycocyanin content [mg cm^{-3}]
V – the volume of solvent [cm^3]
d_w – the dried biomass [g TS].

Results and Discussion

Effects of Light Wavelength on Cell Growth and Biomass Productivity

In the experiment, cyanobacteria *Arthrospira platensis* were cultured using various light sources i.e. a fluorescent lamp and red, blue, and red-blue electroluminescent diodes, and the illuminance of light emitted on the photobioreactor surface was 7500 lux. The initial biomass concentration in all experimental series was 0.369 ± 0.014 g TS dm^{-3}. As shown in Figure 2, the highest biomass concentration was obtained in the culture with blue-red LED lighting and it amounted to 5.619 ± 0.053 g TS dm^{-3}. In terms of biomass concentration, the culture using red LED lighting appeared to be the second highest with a value of 3.915 ± 0.083 g TS dm^{-3}. The lowest biomass concentration of 2.789 ± 0.032 g TS dm^{-3} was noted in the culture lit by the fluorescent lamp (Fig. 2). The experiment results are consistent with a study by LEE et al. (2016). The *Arthrospira platensis* were cultured using three light sources: red LED (660 nm), red-blue LED (660 and 450 nm), and blue LED (450 nm). The obtained results show that the highest biomass concentration of 3.2 g TS dm^{-3} was obtained in the culture using red-blue LED lighting. WANG et al. (2007) and CHEN et al.

(2010) investigated the growth of *Arthrospira platensis* using red, yellow, blue, white and green LED diodes. They observed that the highest biomass concentration is obtained when using a red LED diode. However, they carried out no testing with the simultaneous use of blue and red culture lighting.

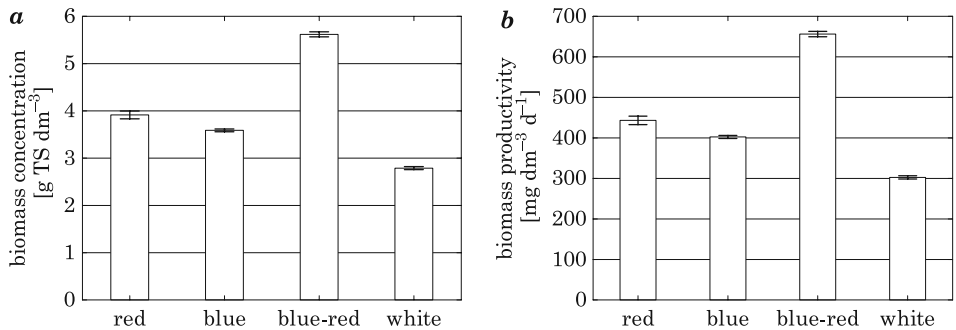

Fig. 2. Effects of light wavelength on: *a* – biomass concentration; *b* – biomass productivity

The values of biomass productivity in particular series were proportional to biomass concentrations. For the culture based on blue-red LED lighting, the biomass productivity was 656.250 ± 6.592 mg TS dm^{-3} d^{-1}, and for a fluorescent lamp it was 302.500 ± 4.005 mg TS dm^{-3} d^{-1} (Fig. 2). A study by Lima et al. (2018) also indicated that cultures using mixed red and blue light exhibit a higher biomass concentration than cultures with a separate red or blue lighting.

Effects of Light Wavelength on Phycocyanin Content and Phycocyanin Productivity of Arthrospira platensis

Figure 3 shows the effect of light of different wavelengths on phycocyanin content and phycocyanin productivity of *Arthrospira platensis*. The highest phycocyanin content was noted for biomass cultured using red LED lighting and it amounted to 17.61 ± 0.51% TS. The culture with blue LED lighting was characterised by the lowest phycocyanin content in the cells, which was 2.47 ± 0.03% TS (Fig. 3). LIMA et al. (2018), when researching the effect of the spectral quality of light on the accumulation of pigments in *Arthrospira platensis* biomass, also observed that the highest phycocyanin concentration (16.71% TS) was obtained with the use of a red LED (660 nm). On the other hand, when a blue light (450 nm) was used, no presence of phycocyanin was demonstrated in *Arthrospira platensis* biomass. CHEN et al. (2010), when researching the effect of red, white, blue, yellow and green LEDs, also observed the highest phycocyanin content of 15.2% TS in the culture using red light.

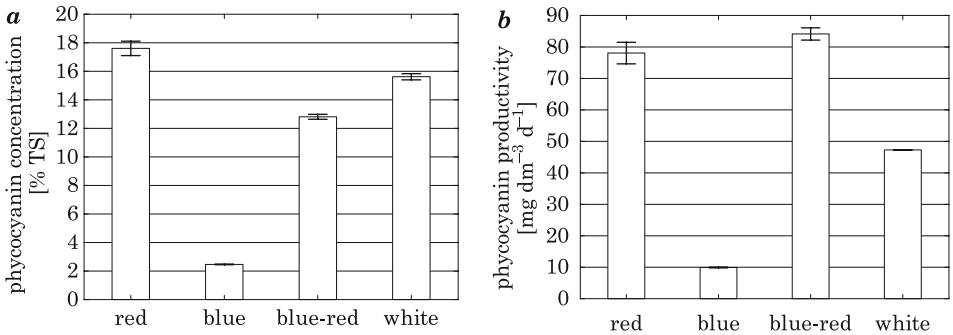

Fig. 3. Effects of light wavelength on: *a* – phycocyanin concentration; *b* – phycocyanin productivity

Despite the highest phycocyanin content in the culture with Red LED lighting, the average phycocyanin productivity was lower by 7.76% compared to the culture using blue-red diodes, which was associated with higher biomass productivity. The highest phycocyanin productivity was 84.12 ± 1.95 mg dm^{-3} d^{-1}. The lowest phycocyanin productivity was recorded in culture using blue LED lighting, which was 9.93 ± 0.14 mg dm^{-3} d^{-1} (Fig. 3).

Effects of Light Wavelength on Purity Grade of Phycocyanin

Phycocyanin purity is determined through the absorbance ratio OD_{615}/OD_{280}, with a purity of ≥ 0.7 regarded as food grade, ≥ 3.9 as reactive grade, and ≥ 4.0 as analytical grade (PATIL et al. 2006). As shown in Table 2, the purity of phycocyanin obtained in the experiment only in the culture using red LED lighting falls in the lowest category with a value of 0.710 ± 0.01. Prior to further uses, phycocyanin from other cultures would have to be purified to the required ranges. WALTER et al. (2011) analysed the production of phycocyanin using various light spectra and also observed that the extract from the culture using a red LED was characterised by the highest purity.

Table 2

The purity grade of phycocyanin in experimental culture

Series	Phycocyanin extraction yield [mg g^{-1} TS]	Phycocyanin purity
Red	176.08 ± 5.08	0.710 ± 0.010
Blue	24.67 ± 0.22	0.251 ± 0.005
Blue-red	128.17 ± 1.75	0.537 ± 0.001
White	156.29 ± 2.18	0.485 ± 0.005

Conclusions

This study concerning the effect of lighting on the intensification of phycocyanin production in a culture of *Arthrospira platensis* demonstrated that the light spectrum affected both an increase in the biomass of the tested cyanobacteria, phycocyanin content of the biomass and the purity of the obtained phycocyanin.

The highest biomass concentration and biomass production efficiency were obtained in a culture using Blue-Red LED lighting and they amounted to 5.619 ± 0.053 g TS dm^{-3} and 656 ± 7 mg dm^{-3} d^{-1}.

The highest phycocyanin concentration and purity were observed in a culture using Red LED lighting and they amounted to 17.61 ± 0.51% TS and 0.710 ± 0.01.

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